

What is claimed is:

1. A time-resolved fluorescence microscope comprising:

a pin hole;

a sample stage on which a sample is mounted;

5 an objective lens system for projecting a reduced-size image of the pin hole on the sample on the sample stage while focusing fluorescence emitted by the sample at the pin hole;

pulsed-laser emitting means;

10 light-dividing means for dividing laser light emitted by the pulsed-laser emitting means into two portions;

means for causing one portion of the laser light divided by the light-dividing means to enter the pin hole as a sample excitation light;

optical-path length varying means for varying the optical path of the other portion of the laser light divided by the light-dividing means;

15 a non-linear optical element for mixing the fluorescence from the sample exiting from the pin hole and the laser light that has passed through the optical-path length varying means to produce a sum frequency light;

spectroscope means for analyzing the sum frequency light produced by the non-linear optical element to spectroscopy;

20 a detector for detecting light emerging from the spectroscope means; and

recording means for recording an output obtained from the detector as the optical path length of the other portion of the laser light is varied by the optical-path length varying means.

25 2. The time-resolved fluorescence microscope according to claim 1, wherein a prism pair is disposed in the optical path of the sample excitation light between the light-dividing means and the pin hole, wherein a negative dispersion is given to the excitation light in advance.

3. The time-resolved fluorescence microscope according to claim 2, further comprising:

a 1/2 wavelength plate disposed in the optical path of the sample excitation light a control unit for controlling; and

5 a control unit for controlling the angle of the 1/2 wavelength plate and the optical-path length varying means in an interlocked manner.

4. The time-resolved fluorescence microscope according to claim 2, wherein a collimator lens, an iris and a condenser lens are disposed between the non-linear
10 optical element and the spectroscopy means, the collimator rendering the light that has passed through the non-linear optical element into parallel light, wherein the sum-frequency light produced by the non-linear optical element is separated before being incident on the spectroscopy means, the up-converted fluorescence signal also go through on optical band-pass filter which is put at the entrance of
15 spectroscopy to make only the up-conversion signal enter the spectroscopy.

5. The time-resolved fluorescence microscope according to claim 4, wherein a total reflection mirror is disposed between the pin hole and the objective lens system as an optical element for bending optical path.